

1

CELL ANALYSIS AND SORTING APPARATUS FOR MANIPULATION OF CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provision application No. 60/164,643, which was filed on Nov. 10, 1999.

FIELD OF THE INVENTION

This invention relates to cell analysis and sorting devices and methods for manipulating cells using these devices. More particularly, the invention relates to a cell analysis and sorting apparatus that can capture and hold single cells at known locations and then selectively release certain of these cells. A method of manipulating the cells using the cell analysis and sorting apparatus is also provided.

BACKGROUND OF THE INVENTION

Many recent technological advances have enhanced the study of cellular biology and biomechanical engineering, most notably by improving methods and devices for carrying out cellular analysis. For example, in the past decade an explosion in the number of optical probes available for cell analysis has enabled an increase in the amount of information gleaned from microscopic and flow cytometric assays. Microscopic assays allow the researcher to monitor the time-response of a limited number of cells using optical probes. Flow cytometry, on the other hand, uses optical probes for assays on statistically significant quantities of cells for sorting into subpopulations.

However, these mechanisms alone are insufficient for time-dependent analysis. Microscopic assays can only track a few cells over time, and do not allow the user to track the location of individual cells. With flow cytometry, the user can only observe each cell once, and can only easily sort a cell population into three subpopulations. Flow cytometry techniques fail to provide for analysis of the same cell multiple times, or for arbitrary sorting of subpopulations. These kinds of bulk assay techniques produce mean statistics, but cannot provide the researcher with distribution statistics.

Advances in microsystems technology have also influenced many applications in the fields of cell biology and biomedical engineering. Scaling down to the micron level allows the use of smaller sample sizes than those used in conventional techniques. Additionally, the smaller size and ability to make large arrays of devices enables multiple processes to be run in parallel.

Integrated circuits have been fabricated on silicon chips since the 1950s, and as processing techniques improve, the size of transistors continues to shrink. The ability to produce large numbers of complex devices on a single chip sparked interest in fabricating mechanical structures on silicon as well. The range of applications for micro electromechanical systems (MEMS) is enormous. Accelerometers, pressure sensors, and actuators are just a few of the many MEMS devices currently produced. Another application of MEMS is in biology and medicine. Micromachined devices have been made for use in drug-delivery, DNA analysis, diagnostics, and detection of cell properties.

Manipulation of cells is another application of MEMS. For example, in the early 1990's, Sato et al. described in his paper, which is hereby incorporated by reference, *Individual and Mass Operation of Biological Cells using Microme-*

2

chanical Silicon Devices, Sensors and Actuators, 1990, A21-A23:948-953, the use of pressure differentials to hold cells. Sato et al. microfabricated hydraulic capture chambers that were used to capture plant cells for use in cell fusion experiments. Pressure differentials were applied so that single cells were sucked down to plug an array of holes. Cells could not be individually released from the array, however, because the pressure differential was applied over the whole array, not to individual holes.

Bousse et al. in his paper, which is hereby incorporated by reference, *Micromachined Multichannel Systems for the Measurement of Cellular Metabolism*, Sensors and Actuators B, 1994, 20:145-150, described arrays of wells etched into silicon to passively capture cells by gravitational settling. Multiple cells were allowed to settle into each of an array of wells where they were held against flow due to the hydrodynamics resulting from the geometry of the wells. Changes in the pH of the medium surrounding the cells were monitored by sensors in the bottom of the wells, but the wells lacked a cell-release mechanism, and multiple cells were trapped in each well. Another known method of cell capture is dielectrophoresis (DEP). DEP refers to the action of neutral particles in non-uniform electric fields. Neutral polarizable particles experience a force in non-uniform electric fields which propels them toward the electric field maxima or minima, depending on whether the particle is more or less polarizable than the medium it is in. By arranging the electrodes properly, an electric field may be produced to stably trap dielectric particles.

Micromachining has been utilized to make electrode arrays for cell manipulation since the late 1980s. Researchers have successfully trapped many different cell types, including mammalian cells, yeast cells, plant cells, and polymeric particles. Much work involves manipulating cells by exploiting differences in the dielectric properties of varying cell types to evoke separations, such as separation of viable from non-viable yeast, and enrichment of CD34+ stem cells from bone marrow and peripheral blood stem cells. More relevant work on trapping cells in various two- and three-dimensional microfabricated electrode geometries has been shown by several groups. However, trapping arrays of cells with the intention of releasing selected subpopulations of cells has not yet been widely explored. Additionally, DEP can potentially induce large temperature changes, causing not only convection effects but also profoundly affecting cell physiology.

These studies demonstrate that it is possible to trap individual and small numbers of cells in an array on a chip, but without the ability to subsequently manipulate and selectively release individual cells. This inability to select or sort based on a biochemical measurement poses a limitation to the kinds of scientific inquiry that may be of interest.

The currently available mechanisms for carrying out cell analysis and sorting are thus limited in their applications. There is thus a need for an improved method and apparatus for sorting and releasing large quantities of cells that can easily and efficiently be used. In addition, there is a need for an analysis and sorting device that allows the user to look at each cell multiple times, and to track many cells over time. Finally, there is a need for a cell sorter that lets the user know the cell locations, and to be able to hold and selectively release the cells so that the user can arbitrarily sort based on any aspect of the cells' characteristic during time-responsive assays.

SUMMARY OF THE INVENTION

The present invention provides a cell sorting apparatus that is capable of monitoring over time the behavior of each